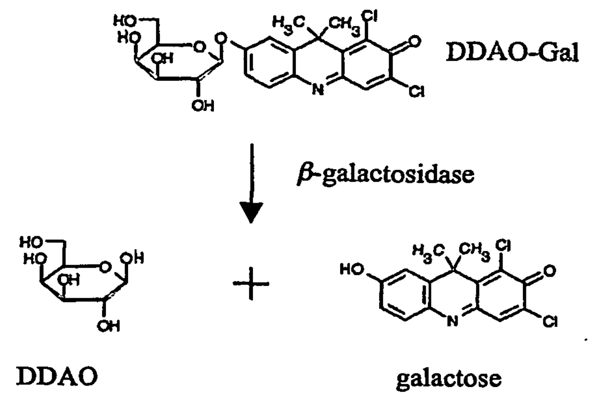
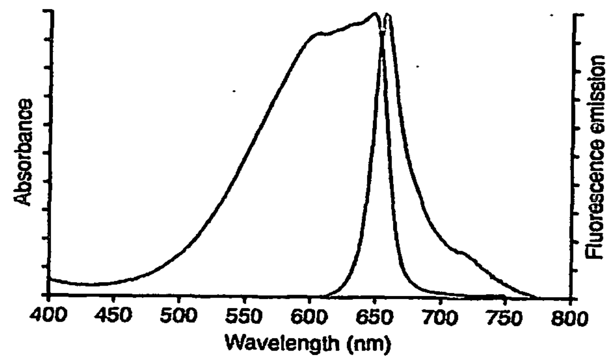
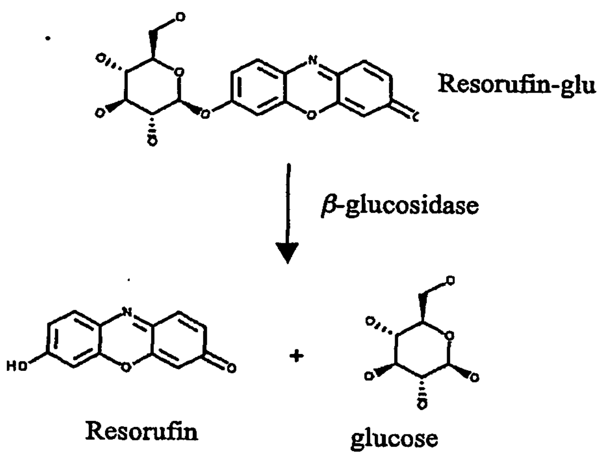

DDAO-Gal
β-galactosidase
DDAO
galactose


Absorbance
Fluorescence emission
400 450 500 550 600 650 700 750 800
Wavelength (nm)


Resorufin-glu
β-glucosidase
Resorufin
glucose

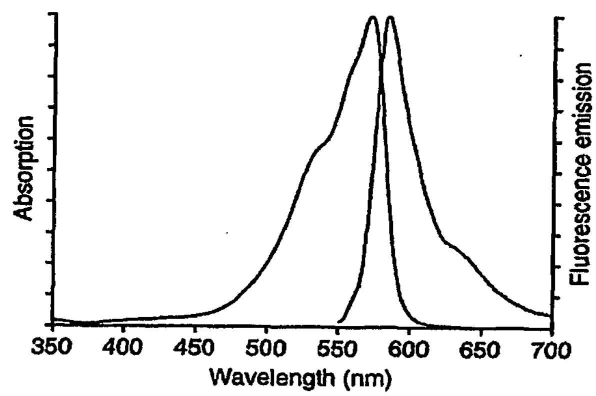
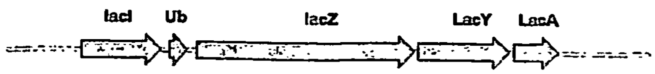

Absorption
Fluorescence emission
350 400 450 500 550 600 650 700
Wavelength (nm)

FIG. 1

(a)



LacOperon Ub-Arg-

(b)

```
                                   M  Q  I  F  V  K  T  L  T  Q  K  T  I  T  L  E  V  E  S  S  D  T  I  D·
CGGATAACAA TTTCACACAG GAAACAGCTA TGCAGATTTT CGTCAAGACT TTGACCGGTA AAACCATAAC ATTGGAAGTT GAATCTTCCG ATACCATCGA
GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACGTCTAAAA GCAGTTCTGA AACTGGCCAT TTTGGTATTG TAACCTTCAA CTTAGAAGGC TATGGTAGCT
·D N  V  K  S  K  I  Q  D  K  E  G  I  P  P  D  Q  Q  R  L  I  F  A  G  K  Q  L  E  D  G  R  T  L  S
CAACGTTAAG TCGAAAATTC AAGACAAGGA AGGTATCCCT CCAGATCAAC AAAGATTGAT CTTTGCCGGT AAGCAGCTAG AAGACGGTAG AACGCTGTCT
GTTGCAATTC AGCTTTTAAG TTCTGTTCCT TCCATAGGGA GGTCTAGTTG TTTCTAACTA GAAACGGCCA TTCGTCGATC TTCTGCCATC TTGCGACAGA
 D  Y  N  I  Q  K  E  S  T  L  H  L  V  L  R  L  R  G  G  R  H  G  S  G  A  V  L  L  P  V  S  L  V K·
GATTACAACA TTCAGAAGGA GTCCACCTTA CATCTTGTGC TAAGGCTAAG AGGTGGTAGG CACGGATCCG GAGCTTGGCT GTTGCCCGTC TCACTGGTGA
CTAATGTTGT AAGTCTTCCT CAGGTGGAAT GTAGAACACG ATTCCGATTC TCCACCATCC GTGCCTAGGC CTCGAACCGA CAACGGGCAG AGTGACCACT
·K R  K  T  T  L  A  P  N  T  Q  T  A  S  P  R  A  L  A  D  S  L  M  Q  L  A  R  Q  V  S  R  L  N R·
AAAGAAAAAC CACCCTGGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA GCTGGCACGA CAGGTTTCCC GACTTAATCG
TTTCTTTTTG GTGGGACCGC GGGTTATGCG TTTGGCGGAG AGGGGCGCGC AACCGGCTAA GTAATTACGT CGACCGTGCT GTCCAAAGGG CTGAATTAGC
·R L  A  A  H  P  P  F  A  S  V  R  N  S  E  E  A  R  T  D  R  P  S  Q  Q  L  R  S  L  N  G  E  V  R
CCTTGCAGCA CATCCCCCTT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGCA GCCTGAATGG CGAATGGCGC
GGAACGTCGT GTAGGGGGAA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCGT CGGACTTACC GCTTACCGCG
```

FIG. 2

k12-e1

```
 +1                   Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser Asp Thr Ile Asp Asn Val Lys Ser Lys
5001  ACAGGAAACA GCTATGCAGA TTTTCGTCAA GACTTTGACC GGTAAAACCA TAACATTGGA AGTTGAATCT TCCGATACCA TCGACAACGT TAAGTCGAAA
      TGTCCTTTGT CGATACGTCT AAAAGCAGTT CTGAAACTGG CCATTTTGGT ATTGTAACCT TCAACTTAGA AGGCTATGGT AGCTGTTGCA ATTCAGCTTT
 +1   Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys
5101  ATTCAAGACA AGGAAGGTAT CCCTCCAGAT CAACAAAGAT TGATCTTTGC CGGTAAGCAG CTAGAAGACG GTAGAACGCT GTCTGATTAC AACATTCAGA
      TAAGTTCTGT TCCTTCCATA GGGAGGTCTA GTTGTTTCTA ACTAGAAACG GCCATTCGTC GATCTTCTGC CATCTTGCGA CAGACTAATG TTGTAAGTCT
 +1   ?Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Leu His Gly Ser Gly Ala Trp Leu Leu Pro Val Ser Leu Val Thr Gln Leu Asn Arg Leu
5201  AGGAGTCCAC CTTACATCTT GTGCTAAGGC TAAGAGGTGG TTTGCACGGA TCCGGAGCTT GGCTGTTGCC CGTCTCACTG GTTACCCAAC TTAATCGCCT
      TCCTCAGGTG GAATGTAGAA CACGATTCCG ATTCTCCACC AAACGTGCCT AGGCCTCGAA CCGACAACGG GCAGAGTGAC CAATGGGTTG AATTAGCGGA
 +1   ?Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe
5301  TGCAGCACAT CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGCGCTTT
      ACGTCGTGTA GGGGGAAAGC GGTCGACCGC ATTATCGCTT CTCCGGGCGT GGCTAGCGGG AAGGGTTGTC AACGCGTCGG ACTTACCGCT TACCGCGAAA
```

k12-e2

```
 +1                   Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser Asp Thr Ile Asp Asn Val Lys Ser Lys
5001  ACAGGAAACA GCTATGCAGA TTTTCGTCAA GACTTTGACC GGTAAAACCA TAACATTGGA AGTTGAATCT TCCGATACCA TCGACAACGT TAAGTCGAAA
      TGTCCTTTGT CGATACGTCT AAAAGCAGTT CTGAAACTGG CCATTTTGGT ATTGTAACCT TCAACTTAGA AGGCTATGGT AGCTGTTGCA ATTCAGCTTT
 +1   Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys
5101  ATTCAAGACA AGGAAGGTAT CCCTCCAGAT CAACAAAGAT TGATCTTTGC CGGTAAGCAG CTAGAAGACG GTAGAACGCT GTCTGATTAC AACATTCAGA
      TAAGTTCTGT TCCTTCCATA GGGAGGTCTA GTTGTTTCTA ACTAGAAACG GCCATTCGTC GATCTTCTGC CATCTTGCGA CAGACTAATG TTGTAAGTCT
 +1   ?Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Leu His Gly Ser Gly Ala Trp Leu Leu Pro Val Ser Leu Val Lys Arg Lys Thr Thr Leu
5201  AGGAGTCCAC CTTACATCTT GTGCTAAGGC TAAGAGGTGG TTTGCACGGA TCCGGAGCTT GGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT
      TCCTCAGGTG GAATGTAGAA CACGATTCCG ATTCTCCACC AAACGTGCCT AGGCCTCGAA CCGACAACGG GCAGAGTGAC CACTTTTCTT TTTGGTGGGA
 +1   ?Leu Ala Pro Asn Thr Gln Thr Ala Ser Pro Thr Gln Leu Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp
5301  GGCGCCCAAT ACGCAAACCG CCTCTCCTAC CCAACTTAAT CGCCTTGCAG CACATCCCCC TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT
      CCGCGGGTTA TGCGTTTGGC GGAGAGGATG GGTTGAATTA GCGGAACGTC GTGTAGGGGG AAAGCGGTCG ACCGCATTAT CGCTTCTCCG GGCGTGGCTA
```

k12-e3a

```
 +1                   Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser Asp Thr Ile Asp Asn Val Lys Ser Lys
5001  ACAGGAAACA GCTATGCAGA TTTTCGTCAA GACTTTGACC GGTAAAACCA TAACATTGGA AGTTGAATCT TCCGATACCA TCGACAACGT TAAGTCGAAA
      TGTCCTTTGT CGATACGTCT AAAAGCAGTT CTGAAACTGG CCATTTTGGT ATTGTAACCT TCAACTTAGA AGGCTATGGT AGCTGTTGCA ATTCAGCTTT
 +1   Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys
5101  ATTCAAGACA AGGAAGGTAT CCCTCCAGAT CAACAAAGAT TGATCTTTGC CGGTAAGCAG CTAGAAGACG GTAGAACGCT GTCTGATTAC AACATTCAGA
      TAAGTTCTGT TCCTTCCATA GGGAGGTCTA GTTGTTTCTA ACTAGAAACG GCCATTCGTC GATCTTCTGC CATCTTGCGA CAGACTAATG TTGTAAGTCT
 +1   ?Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Leu His Lys Arg Lys Thr Thr Leu Ala Thr Gln Leu Asn Arg Leu Ala Ala His Pro Pro
5201  AGGAGTCCAC CTTACATCTT GTGCTAAGGC TAAGAGGTGG TTTGCACAAA AGAAAAACCA CCCTGGCGAC CCAACTTAAT CGCCTTGCAG CACATCCCCC
      TCCTCAGGTG GAATGTAGAA CACGATTCCG ATTCTCCACC AAACGTGTTT TCTTTTTGGT GGGACCGCTG GGTTGAATTA GCGGAACGTC GTGTAGGGGG
 +1   ?Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe Pro Ala
5301  TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGCCCTTCCC AACAGTTGCG CAGCCTGAAT GGCGAATGGC GCTTTGCCTG GTTTCCGGCA
      AAAGCGGTCG ACCGCATTAT CGCTTCTCCG GGCGTGGCTA GCGGGAAGGG TTGTCAACGC GTCGGACTTA CCGCTTACCG CGAAACGGAC CAAAGGCCGT
```

k12-n3

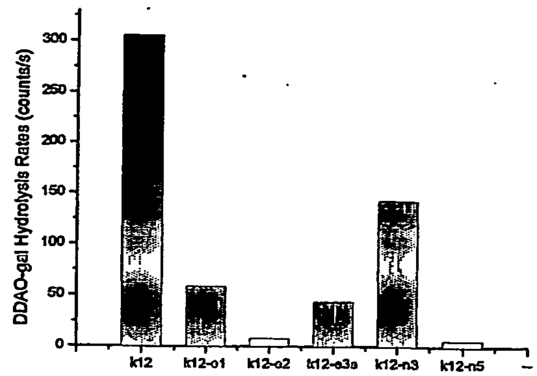
```
 +1                   Met Lys Leu Pro Ile Tyr Leu Asp Tyr Ser Ala Ser Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Arg Asp Trp Glu
5001  ACAGGAAACA GCTATGAAAC TGCCGATTTA TCTGGATTAT AGCGCGAGCA CCATGATTAC GGATTCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA
      TGTCCTTTGT CGATACTTTG ACGGCTAAAT AGACCTAATA TCGCGCTCGT GGTACTAATG CCTAAGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT
 +1   Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu
5101  AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT
      TTGGGACCGC AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA
```

K12-n5

```
 +1                   Met Gln Glu Gly Gln Asn Arg Lys Thr Ser Ser Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Arg Asp Trp Glu
5001  ACAGGAAACA GCTATGATGC AGGAAGGCCA GAACCGCAAA ACCAGCAGCA CCATGATTAC GGATTCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA
      TGTCCTTTGT CGATACTACG TCCTTCCGGT CTTGGCGTTT TGGTCGTCGT GGTACTAATG CCTAAGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT
 +1   Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu
5101  AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT
      TTGGGACCGC AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA
```

FIG 3a

(b)



DDAO-gal Hydrolysis Rates (counts/s)
300
250
200
150
100
50
0
k12
k12-o1
k12-o2
k12-o3a
k12-n3
k12-n5

FIG 3b

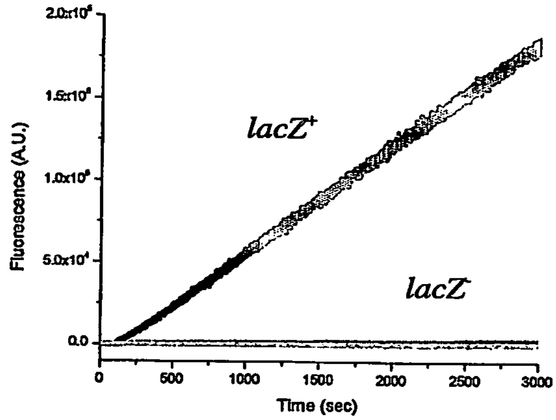
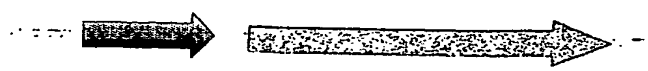


FIG. 4

(a)



(b)

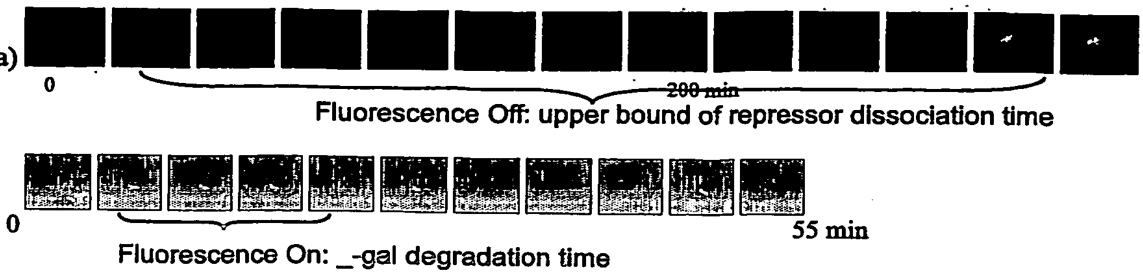
EcoRV

```
      ·I  S  V   V  G  Y  D   D  T  E   D  S  S   C  Y  I  P   P  L  T   T  I  K   Q  D  F  R   L  L  G   Q  T  S
801   TATCTCGGTA GTGGGATACG ACGATACCGA AGACAGCTCA TGTTATATCC CGCCGTTAAC CACCATCAAA CAGGATTTTC GCCTGCTGGG GCAAACCAGC
      ATAGAGCCAT CACCCTATGC TGCTATGGCT TCTGTCGAGT ACAATATAGG GCGGCAATTG GTGGTAGTTT GTCCTAAAAG CGGACGACCC CGTTTGGTCG

       V  D  R  L   L  Q  L   S  Q  G   Q  A  V  K   G  N  Q   L  L  P   V  S  L  V   K  R  K   T  T  L   A  P  N  T·
901   GTGGACCGCT TGCTGCAACT CTCTCAGGGC CAGGCGGTGA AGGGCAATCA GCTGTTGCCC GTCTCACTGG TGAAAAGAAA AACCACCCTG GCGCCCAATA
      CACCTGGCGA ACGACGTTGA GAGAGTCCCG GTCCGCCACT TCCCGTTAGT CGACAACGGG CAGAGTGACC ACTTTTCTTT TTGGTGGGAC CGCGGGTTAT

      · Q  T  A   S  P  R   A  L  A  D   S  L  M   Q  L  A   R  Q  V  S   R  L  E   S  G  Q   ·
1001  CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG
      GCGTTTGGCG GAGAGGGGCG CGCAACCGGC TAAGTAATTA CGTCGACCGT GCTGTCCAAA GGGCTGACCT TTCGCCCGTC ACTCGCGTTG CGTTAATTAC

1101  TGAGTCAGCT CACTCATTAG GCACCCCAGG CTTTACACTT TATGCGTCCG GCTCGTATGT TGTGTGAAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA
      ACTCAGTCGA GTGAGTAATC CGTGGGGTCC GAAATGTGAA ATACGCAGGC CGAGCATACA ACACACTTTA ACACTCGCCT ATTGTTAAAG TGTGTCCTTT

1201  [illegible]

1301  [illegible]
                                                        NspI

1401  [illegible]
```

FIG. 5

FIG. 6

(a)


0
200 min
Fluorescence Off: upper bound of repressor dissociation time
0
55 min
Fluorescence On: _-gal degradation time

(b)

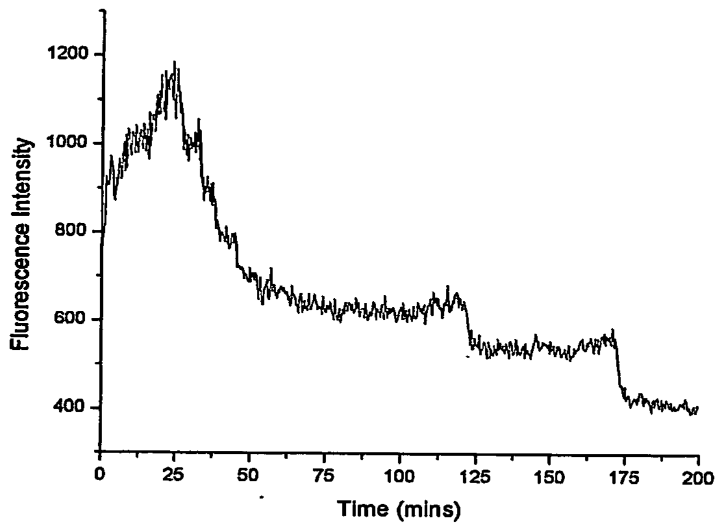
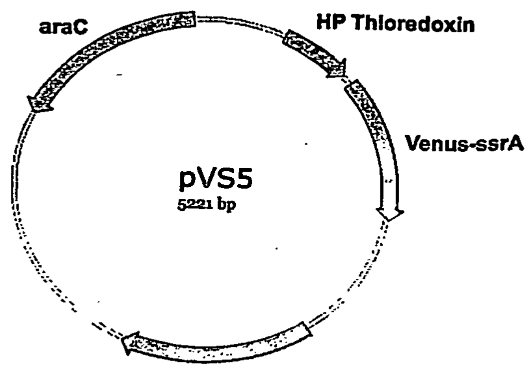

Fluorescence Intensity
1200
1000
800
600
400
0
25
50
75
100
125
150
175
200
Time (mins)

FIG. 7

(a)



(b)

```
                                            Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys
ACAAGCTGGG AATTGATCCC TTCACCAGCA AGGGCGAGGA GCTGTTCACC GGGGTGGTGC CCATCCTGGT CGAGCTGGAC GGCGACGTAA ACGGCCACAA
TGTTCGACCC TTAACTAGGG AAGTGGTCGT TCCCGCTCCT CGACAAGTGG CCCCACCACG GGTAGGACCA GCTCGACCTG CCGCTGCATT TGCCGGTGTT
Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Leu Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
GTTCAGCGTG TCCGGCGAGG GCGAGGGCGA TGCCACCTAC GGCAAGCTGA CCCTGAAGCT GATCTGCACC ACCGGCAAGC TGCCCGTGCC CTGGCCCACC
CAAGTCGCAC AGGCCGCTCC CGCTCCCGCT ACGGTGGATG CCGTTCGACT GGGACTTCGA CTAGACGTGG TGGCCGTTCG ACGGGCACGG GACCGGGTGG
Leu Val Thr Thr Leu Gly Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val-
CTCGTGACCA CCCTGGGCTA CGGCCTGCAG TGCTTCGCCC GCTACCCCGA CCACATGAAG CAGCACGACT TCTTCAAGTC CGCCATGCCC GAAGGCTACG
GAGCACTGGT GGGACCCGAT GCCGGACGTC ACGAAGCGGG CGATGGGGCT GGTGTACTTC GTCGTGCTGA AGAAGTTCAG GCGGTACGGG CTTCCGATGC
-Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys-
TCCAGGAGCG CACCATCTTC TTCAAGGACG ACGGCAACTA CAAGACCCGC GCCGAGGTGA AGTTCGAGGG CGACACCCTG GTGAACCGCA TCGAGCTGAA
AGGTCCTCGC GTGGTAGAAG AAGTTCCTGC TGCCGTTGAT GTTCTGGGCG CGGCTCCACT TCAAGCTCCC GCTGTGGGAC CACTTGGCGT AGCTCGACTT
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
GGGCATCGAC TTCAAGGAGG ACGGCAACAT CCTGGGGCAC AAGCTGGAGT ACAACTACAA CAGCCACAAC GTCTATATCA CCGCCGACAA GCAGAAGAAC
CCCGTAGCTG AAGTTCCTCC TGCCGTTGTA GGACCCCGTG TTCGACCTCA TGTTGATGTT GTCGGTGTTG CAGATATAGT GGCGGCTGTT CGTCTTCTTG
Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val-
GGCATCAAGG CCAACTTCAA GATCCGCCAC AACATCGAGG ACGGCGGCGT GCAGCTCGCC GACCACTACC AGCAGAACAC CCCCATCGGC GACGGCCCCG
CCGTAGTTCC GGTTGAAGTT CTAGGCGGTG TTGTAGCTCC TGCCGCCGCA CGTCGAGCGG CTGGTGATGG TCGTCTTGTG GGGGTAGCCG CTGCCGGGGC
-Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala-
TGCTGCTGCC CGACAACCAC TACCTGAGCT ACCAGTCCGC CCTGAGCAAA GACCCCAACG AGAAGCGCGA TCACATGGTC CTGCTGGAGT TCGTGACCGC
ACGACGACGG GCTGTTGGTG ATGGACTCGA TGGTCAGGCG GGACTCGTTT CTGGGGTTGC TCTTCGCGCT AGTGTACCAG GACGACCTCA AGCACTGGCG
Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Ala Ala Asn Asp Glu Asn Tyr Ala Leu Ala Ala ***
CGCCGGGATC ACTCTCGGCA TGGACGAGCT GTACAAGGCC GCCAACGACG AGAACTACGC CTTAGCCGCC TAAGAAAAGG GCGAGCTCAA GCTTGAAGGT
GCGGCCCTAG TGAGAGCCGT ACCTGCTCGA CATGTTCCGG CGGTTGCTGC TCTTGATGCG GAATCGGCGG ATTCTTTTCC CGCTCGAGTT CGAACTTCCA
```

FIG. 8

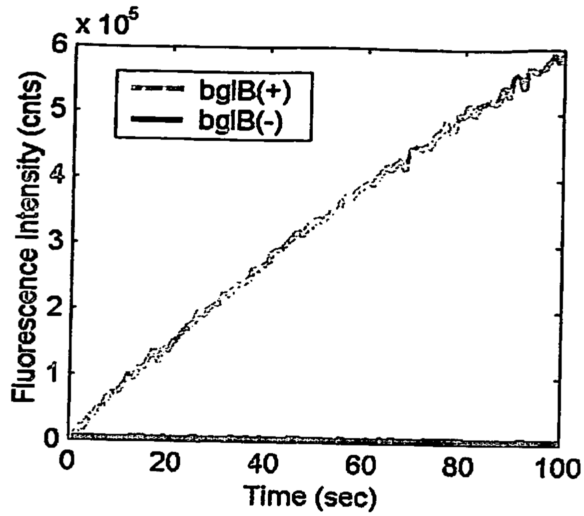

x 10^5
Fluorescence Intensity (cnts)
bglB(+)
bglB(-)
0
1
2
3
4
5
6
0
20
40
60
80
100
Time (sec)

FIG. 9

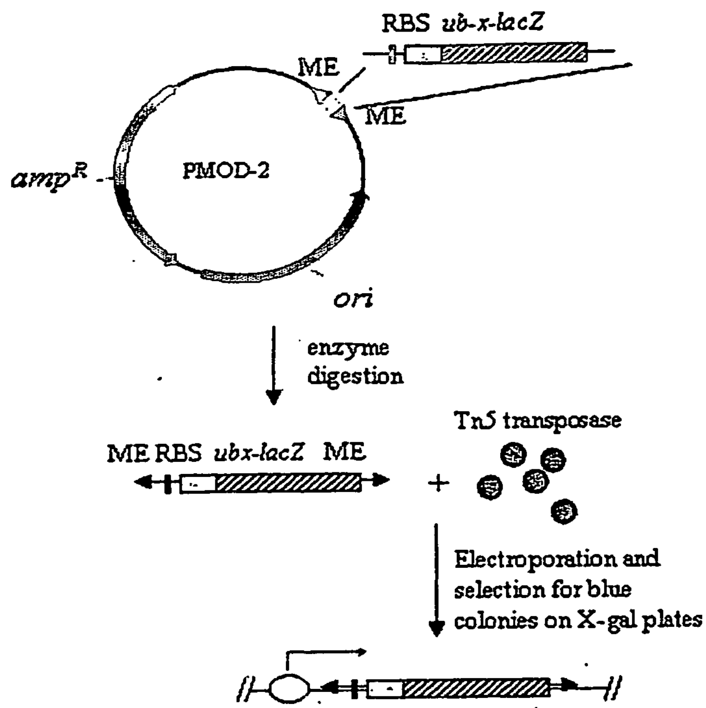

RBS ub-x-lacZ
ME
ME
ampR
PMOD-2
ori
enzyme digestion
Tn5 transposase
ME RBS ubx-lacZ ME
+
Electroporation and selection for blue colonies on X-gal plates

FIG. 10

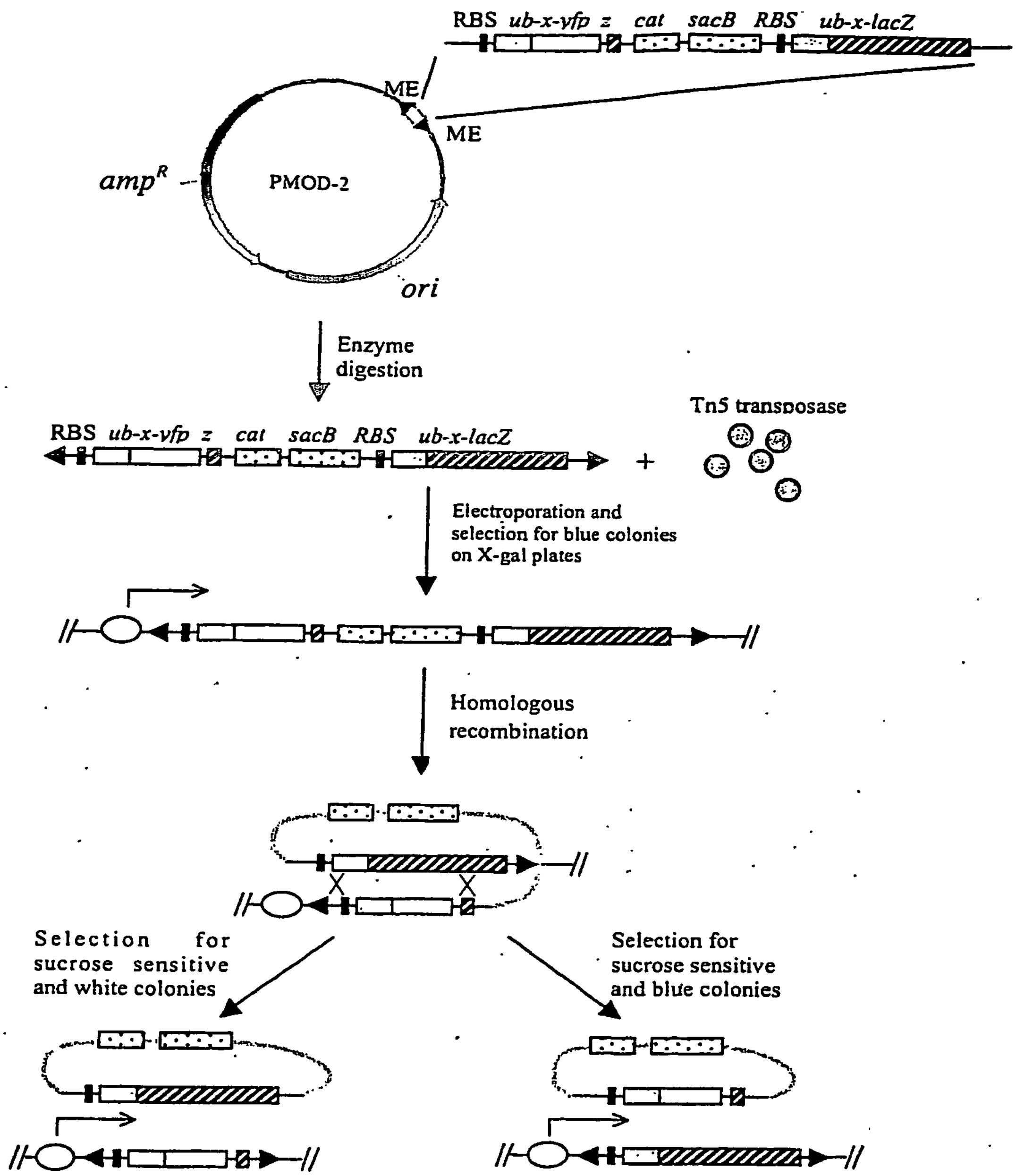

RBS ub-x-yfp z cat sacB RBS ub-x-lacZ
ME
ME
amp^R
PMOD-2
ori
Enzyme digestion
RBS ub-x-yfp z cat sacB RBS ub-x-lacZ
+
Tn5 transposase
Electroporation and selection for blue colonies on X-gal plates
Homologous recombination
Selection for sucrose sensitive and white colonies
Selection for sucrose sensitive and blue colonies

FIG. 11

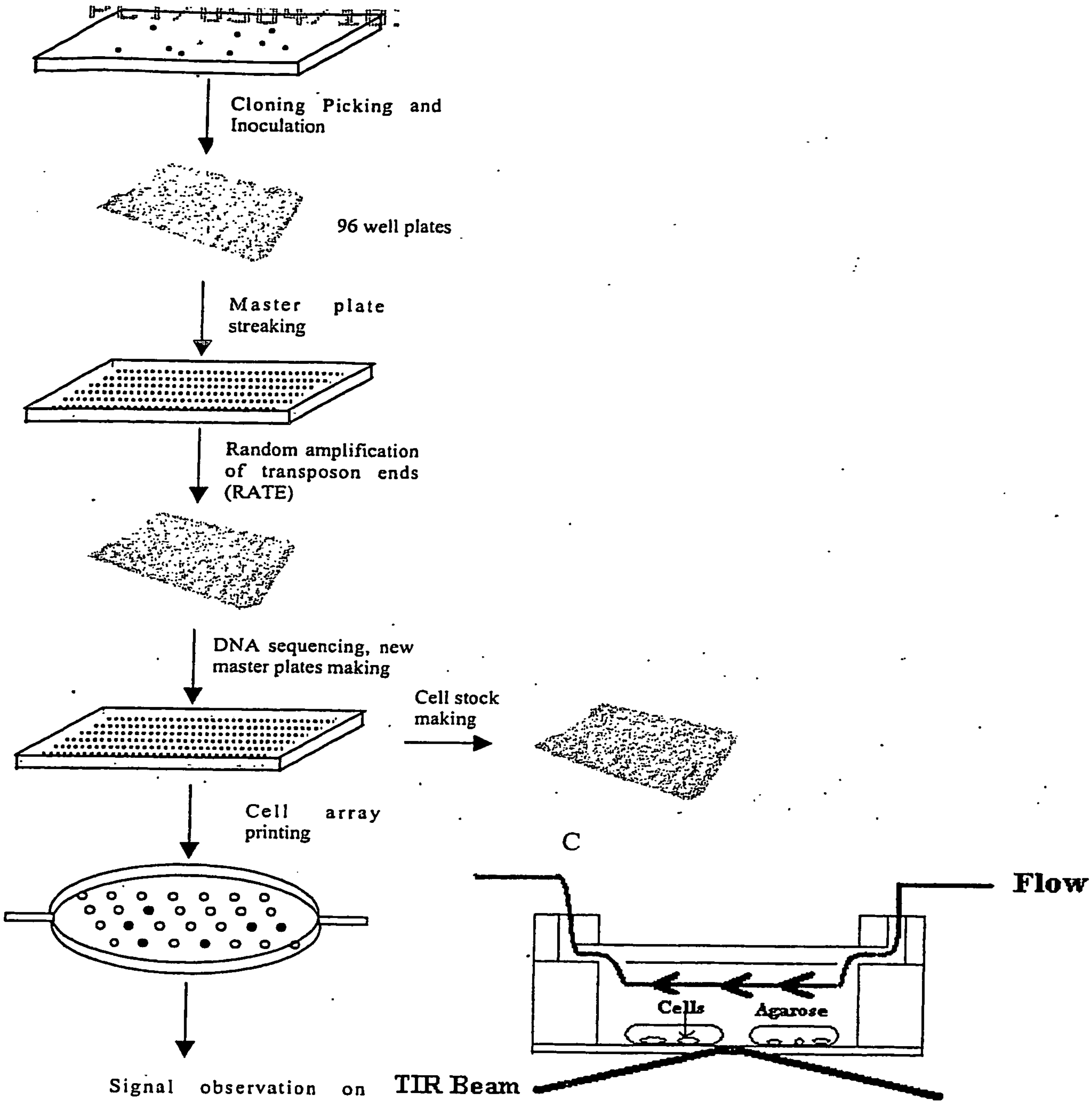
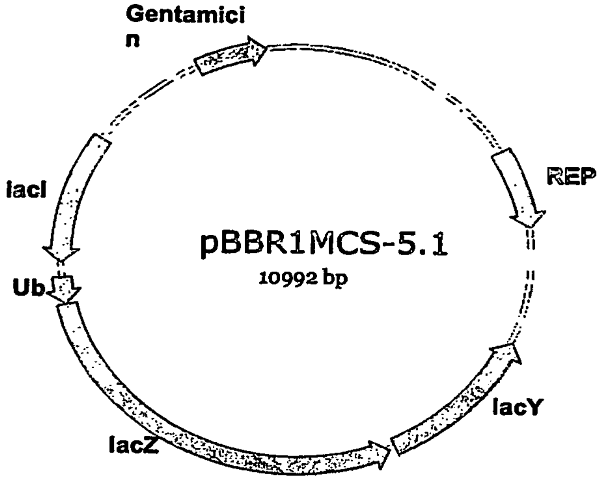

Cloning Picking and Inoculation
96 well plates
Master plate streaking
Random amplification of transposon ends (RATE)
DNA sequencing, new master plates making
Cell stock making
Cell array printing
Signal observation on
C
Flow
Cells
Agarose
TIR Beam

FIG. 12

(a)


Gentamici
n
REP
lacI
pBBR1MCS-5.1
10992 bp
Ub
lacY
lacZ

(b)

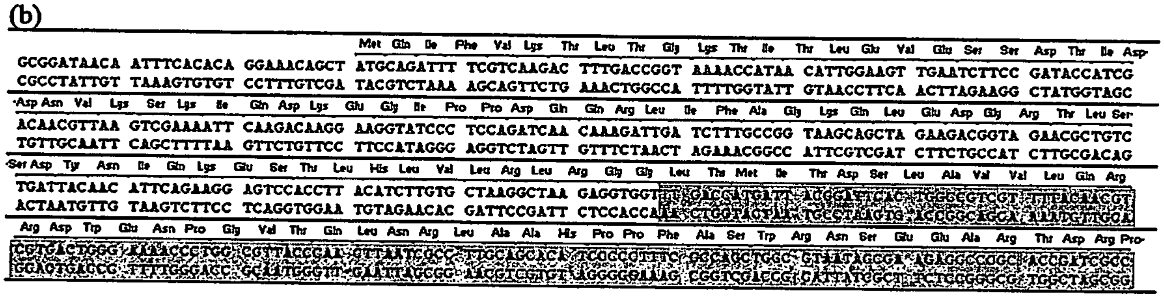

Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser Asp Thr Ile Asp
GCGGATAACA ATTTCACACA GGAAACAGCT ATGCAGATTT TCGTCAAGAC TTTGACCGGT AAAACCATAA CATTGGAAGT TGAATCTTCC GATACCATCG
CGCCTATTGT TAAAGTGTGT CCTTTGTCGA TACGTCTAAA AGCAGTTCTG AAACTGGCCA TTTTGGTATT GTAACCTTCA ACTTAGAAGG CTATGGTAGC
Asp Asn Val Lys Ser Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ser
ACAACGTTAA GTCGAAAATT CAAGACAAGG AAGGTATCCC TCCAGATCAA CAAAGATTGA TCTTTGCCGG TAAGCAGCTA GAAGACGGTA GAACGCTGTC
TGTTGCAATT CAGCTTTTAA GTTCTGTTCC TTCCATAGGG AGGTCTAGTT GTTTCTAACT AGAAACGGCC ATTCGTCGAT CTTCTGCCAT CTTGCGACAG
Ser Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Leu Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg
TGATTACAAC ATTCAGAAGG AGTCCACCTT ACATCTTGTG CTAAGGCTAA GAGGTGGT
ACTAATGTTG TAAGTCTTCC TCAGGTGGAA TGTAGAACAC GATTCCGATT CTCCACCA
Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp Arg Pro

FIG. 13

(a)

Figure 1. Fluorescence image of *Shewanella oneideinsis* cells containing *lacZ* plasmid taken with a through-lens total internal reflection (TIR) fluorescence microscope. Each bright spot is a single cell, in which DDAO generated by the basal level expression of β-gal is detected with high sensitivity.

(b)

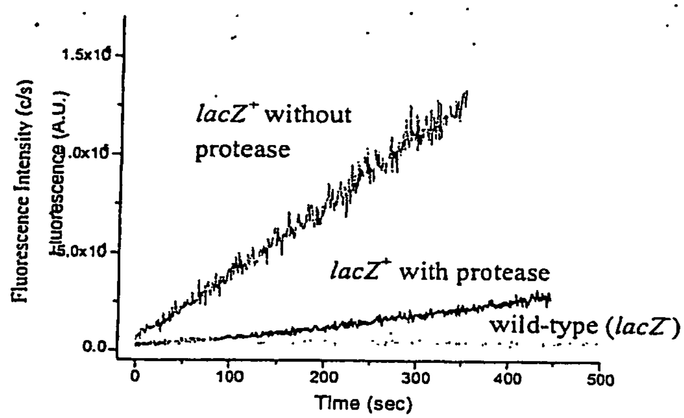
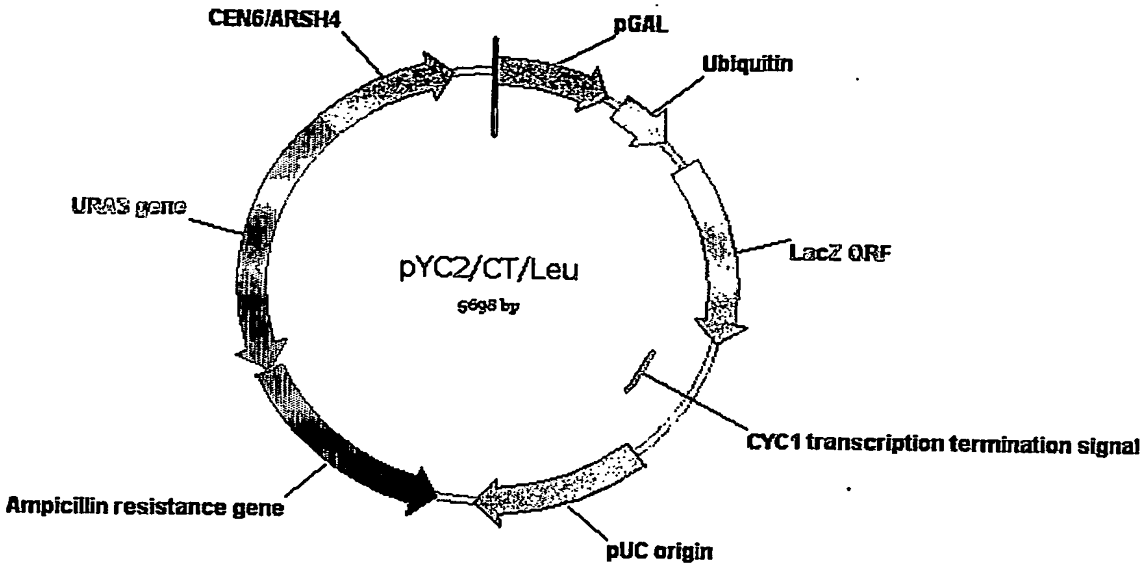


Figure 2. DDAO fluorescence generated by hydrolysis of DDAO-Gal with the wild-type *Shewanella oneideinsis* cells (light gray lines), with cells containing *lacZ* plasmid (gray lines), and cells containing both *lacZ* and the ubiquitin-specific protease plasmid (black lines).

FIG 14

FIG. 1 (a)



(b)

```
   1  ACGGATTAGA AGCCGCCGAG CGGGTGACAG CCCTCCGAAG GAAGACTCTC CTCCGTGCGT CCTCGTCTTC ACCGGTCGCG
      TGCCTAATCT TCGGCGGCTC GCCCACTGTC GGGAGGCTTC CTTCTGAGAG GAGGCACGCA GGAGCAGAAG TGGCCAGCGC
  81  TTCCTGAAAC GCAGATGTGC CTCGCGCCGC ACTGCTCCGA ACAATAAAGA TTCTACAATA CTAGCTTTTA TGGTTATGAA
      AAGGACTTTG CGTCTACACG GAGCGCGGCG TGACGAGGCT TGTTATTTCT AAGATGTTAT GATCGAAAAT ACCAATACTT
 161  GAGGAAAAAT TGGCAGTAAC CTGGCCCCAC AAACCTTCAA ATGAACGAAT CAAATTAACA ACCATAGGAT GATAATGCGA
      CTCCTTTTTA ACCGTCATTG GACCGGGGTG TTTGGAAGTT TACTTGCTTA GTTTAATTGT TGGTATCCTA CTATTACGCT
 241  TTAGTTTTTT AGCCTTATTT CTGGGGTAAT TAATCAGCGA AGCGATGATT TTTGATCTAT TAACAGATAT ATAAATGCAA
      AATCAAAAAA TCGGAATAAA GACCCCATTA ATTAGTCGCT TCGCTACTAA AAACTAGATA ATTGTCTATA TATTTACGTT
 321  AAACTGCATA ACCACTTTAA CTAATACTTT CAACATTTTC GGTTTGTATT ACTTCTTATT CAAATGTAAT AAAAGTATCA
      TTTGACGTAT TGGTGAAATT GATTATGAAA GTTGTAAAAG CCAAACATAA TGAAGAATAA GTTTACATTA TTTTCATAGT
 401  ACAAAAAATT GTTAATATAC CTCTATACTT TAACGTCAAG GAGAAAAAAC CCCGGATCGG ACTACTAGCA GCTGTAATAC
      TGTTTTTTAA CAATTATATG GAGATATGAA ATTGCAGTTC CTCTTTTTTG GGGCCTAGCC TGATGATCGT CGACATTATG
  +3                                    Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu
 481  GACTCACTAT AGGGAATATT AAGCTTGGTA CCATGCAGAT TTTCGTCAAG ACTTTGACCG GTAAAACCAT AACATTGGAA
      CTGAGTGATA TCCCTTATAA TTCGAACCAT GGTACGTCTA AAAGCAGTTC TGAAACTGGC CATTTTGGTA TTGTAACCTT
  +3  Val Glu Ser Ser Asp Thr Ile Asp Asn Val Lys Ser Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu
 561  GTTGAATCTT CCGATACCAT CGACAACGTT AAGTCGAAAA TTCAAGACAA GGAAGGTATC CCTCCAGATC AACAAAGATT
      CAACTTAGAA GGCTATGGTA GCTGTTGCAA TTCAGCTTTT AAGTTCTGTT CCTTCCATAG GGAGGTCTAG TTGTTTCTAA
  +3  Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val
 641  GATCTTTGCC GGTAAGCAGC TAGAAGACGG TAGAACGCTG TCTGATTACA ACATTCAGAA GGAGTCCACC TTACATCTTG
      CTAGAAACGG CCATTCGTCG ATCTTCTGCC ATCTTGCGAC AGACTAATGT TGTAAGTCTT CCTCAGGTGG AATGTAGAAC
  +3  Val Leu Arg Leu Arg Gly Gly Leu His Gly Ser Gly Ala Trp Leu Leu Pro Val Ser Leu Val Lys Arg Lys Thr Thr Leu
 721  TGCTAAGGCT AAGAGGTGGT TTGCACGGAT CCGGAGCTTG GCTGTTGCCC GTCTCACTGG TGAAAAGAAA AACCACCCTG
      ACGATTCCGA TTCTCCACCA AACGTGCCTA GGCCTCGAAC CGACAACGGG CAGAGTGACC ACTTTTCTTT TTGGTGGGAC
  +3  Ala Pro Asn Thr Gln Thr Ala Ser Pro Arg Ala Leu Ala Asp Ser Leu Met Gln Leu Ala Arg Gln Val Ser Arg Leu Asn
 801  GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT CCCGACTTAA
      CGCGGGTTAT GCGTTTGGCG GAGAGGGGCG CGCAACCGGC TAAGTAATTA CGTCGACCGT GCTGTCCAAA GGGCTGAATT
  +3  Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg
 881  TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG CCCGCACCGA TCGCCCTTCC CAACAGTTGC
      AGCGGAACGT CGTGTAGGGG GAAAGCGGTC GACCGCATTA TCGCTTCTCC GGGCGTGGCT AGCGGGAAGG GTTGTCAACG
  +3  Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Glu Ser Trp Leu Glu Cys Asp Leu
 961  GCAGCCTGAA TGGCGAATGG CGCTTTGCCT GGTTTCCGGC ACCAGAAGCG GTGCCGGAAA GCTGGCTGGA GTGCGATCTT
      CGTCGGACTT ACCGCTTACC GCGAAACGGA CCAAAGGCCG TGGTCTTCGC CACGGCCTTT CGACCGACCT CACGCTAGAA
  +3  Pro Glu
1041  CCTGAGG
      GGACTCC
```

FIG. 15

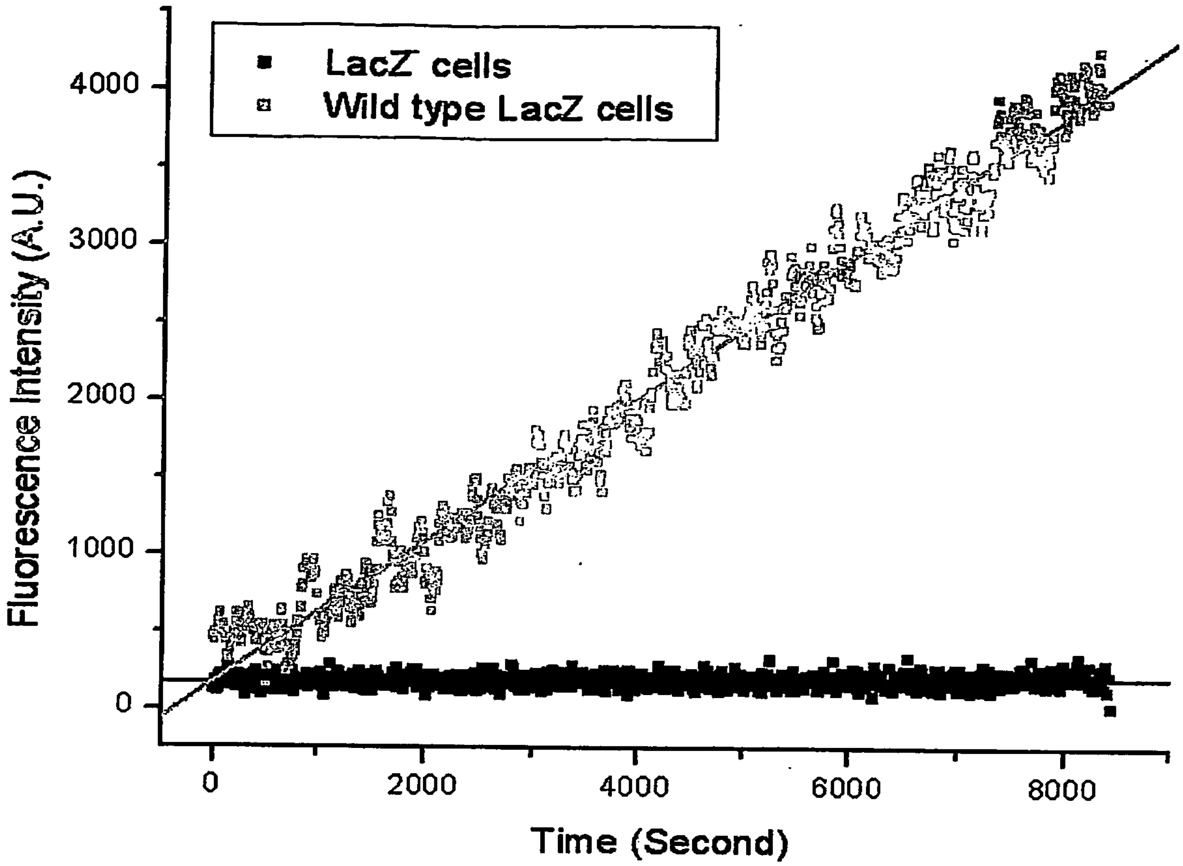

LacZ⁻ cells
Wild type LacZ cells
Fluorescence Intensity (A.U.)
0
1000
2000
3000
4000
0
2000
4000
6000
8000
Time (Second)

FIG. 16

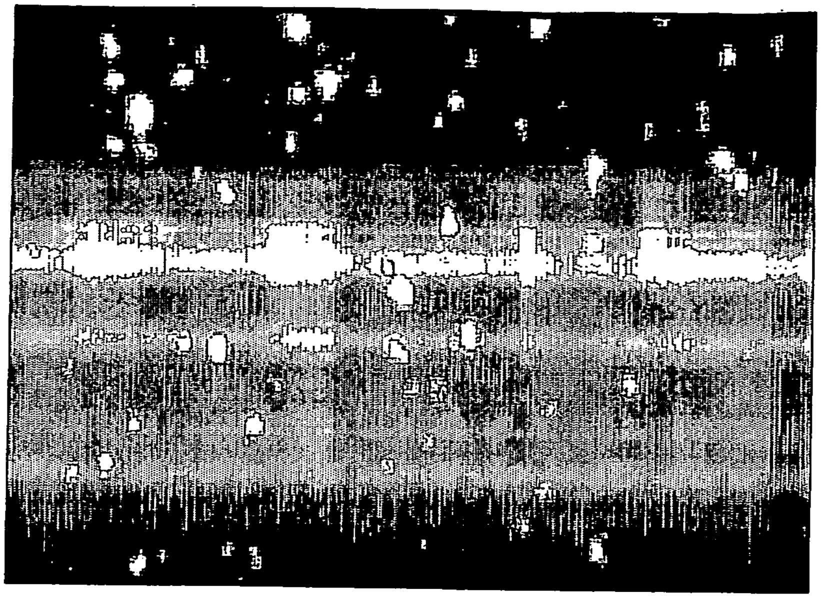


FIG. 17

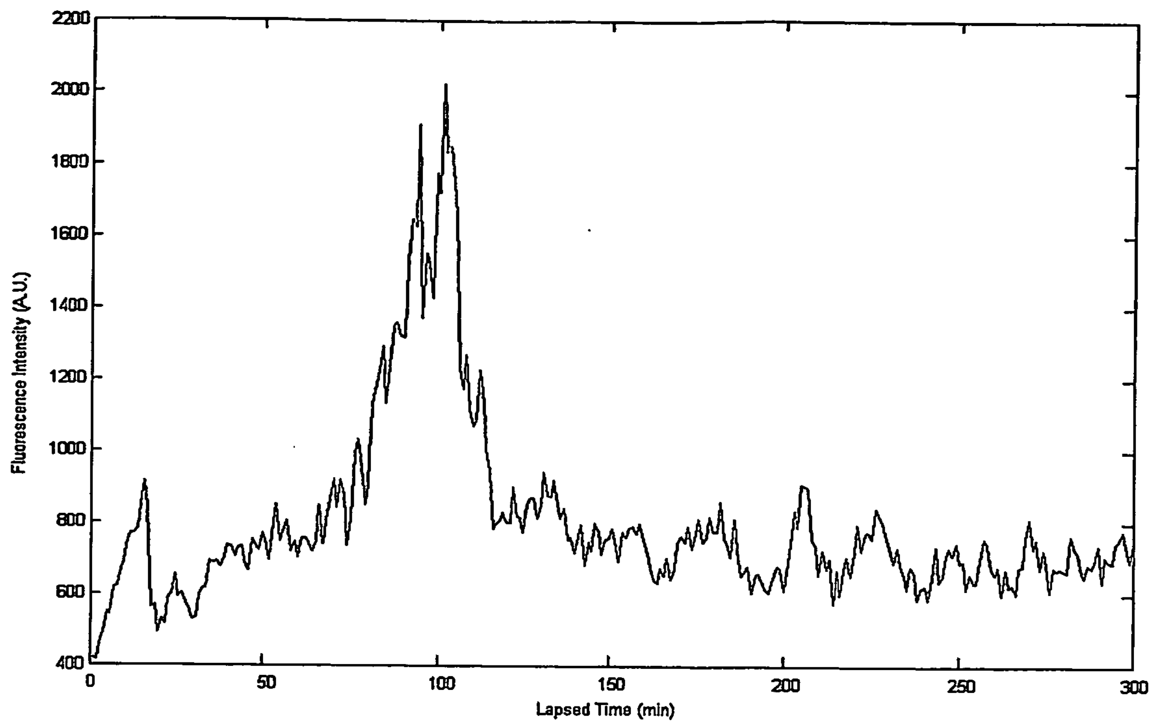

2200
2000
1800
1600
1400
1200
1000
800
600
400
Fluorescence Intensity (A.U.)
0
50
100
150
200
250
300
Lapsed Time (min)

FIG. 18